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Dermal Absorption and Pharmacokinetics of Pesticides in Rats

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16. ABSTRACT

Dermal absorption and disposition of carbaryl, carbofuran, chlordecone, hexachlorobiphenyl and dinoseb were studied in female Fischer 344 rats. Acetone solution on the [^{14}C] labeled pesticide was applied to a previously clipped mid-dorsal region of the back. At various time intervals, animals were killed and selected organs were analyzed for radioactive [^{14}C] content. A physiological compartmental model was fitted to the skin penetration, tissue distribution, and excretion data. The equilibrium tissue to blood ratios and excretion rate constants and skin absorption rate were determined from the model. In many cases, differences in kinetics and retention were found between young and adult rats. The dermal penetration function was single or biexponential, requiring a one or two compartmental model for simulating the skin penetration process.

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Chapter 14

Dermal Absorption and Pharmacokinetics of Pesticides in Rats

P. V. Shah[1], H. L. Fisher[2], M. R. Sumler[3], and L. L. Hall[2]

[1]Toxic Effects Branch, Health and Environmental Review Division (TS-796), Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC 20460
[2]Cellular Biology Branch, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711
[3]Northrop Environmental Sciences, Northrop Corporation, Research Triangle Park, NC 27709

Dermal absorption and disposition of carbaryl, carbofuran, chlordecone, hexachlorobiphenyl and dinoseb were studied in female Fischer 344 rats. Acetone solution of the [^{14}C] labeled pesticide was applied to a previously clipped mid-dorsal region of the back. At various time intervals, animals were killed and selected organs were analyzed for radioactive [^{14}C] content. A physiological compartmental model was fitted to the skin penetration, tissue distribution, and excretion data. The equilibrium tissue to blood ratios and excretion rate constants and skin absorption rate were determined from the model. In many cases, differences in kinetics and retention were found between young and adult rats. The dermal penetration function was single or biexponential, requiring a one or two compartmental model for simulating the skin penetration process.

More than one billion pounds of pesticides are being used each year in the United States alone to increase the productivity of agricultural land (1). Greater reliance on use of pesticides appears inevitable as the demand for food production increases with increasing population. Occupational exposure to pesticides occurs via oral, dermal, and inhalation routes. Although the rate of absorption may be higher by pulmonary and oral routes, the total amount absorbed after skin contact may be much greater during occupational exposure to pesticides (2,3).

Knowledge of dermal absorption (rates and extent) of pesticides is very important in risk assessment and establishment

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of reentry standards for field workers. With regard to occupational exposure to toxicants in general, risk assessment has usually been based on studies in adults. Skin absorption depends on a number of factors, including the chemical and physical nature of the penetrant and type of skin. The limited literature on the effects of age on skin penetration has been recently summarized (4). It appears that the effect of age on percutaneous absorption varies with species, compound, and method of assessment. Increased skin permeability of neosynephrine (as measured by the blanching response) in premature infants compared to full term infants has been documented (5).

No differences in percutaneous absorption of testosterone between the newborn and adult rhesus monkey was noted (6), while more rapid loss of topically applied triadimefon from the skin of young Sprague-Dawley rats has been reported (7). A recent comparative study on percutaneous absorption of pesticides in young and adult Fischer 344 rats reported age-dependent percutaneous absorption for 11 out of 14 pesticides studied (8).

We present here the comparative percutaneous absorption and disposition of carbaryl, carbofuran, chlordecone, hexachlorobiphenyl, and dinoseb in young and adult Fischer 344 rats utilizing identical methodology. Also, comparison of the results of in vivo and two in vitro methods for percutaneous absorption measurements are presented. The utility of the physiological compartmental model in simulating dermal absorption and disposition is discussed.

EXPERIMENTAL APPROACH:

CHEMICALS

The chemicals studied, their respective specific activities, radiolabeling position, and sources are listed in Table I.

The radiochemical purity was greater than 98% for all pesticides studied. Unlabeled chemicals were supplied by the Pesticide and Industrial Chemical Repository (U.S. Environmental Protection Agency, Research Triangle Park, NC). Minimum Essential Medium (MEM) Eagle Formula with Earl's salts and L- glutamine and without phenol red and sodium carbonate was purchased from Gibco Laboratories, Chagrin Falls, OH. HEPES (*N*- 2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), sodium pyruvate, L- serine, L- aspartic acid, and gentamicin were purchased from Gibco, Grand Island, NY. Anhydrous sodium carbonate, sodium hydroxide, ethyl ether (anhydrous), and acetone (reagent grade) were purchased from Fisher Scientific Co., Fairlawn, NJ.

IN VIVO APPLICATION

Female Fischer 344 rats of 33 (young) and 82 (adult) days of age were utilized for dermal absorption experiments. Three rats were used for each time point. Randomized offspring from timed-pregnant rats were used in order to have better control over the age and weight of the animals used in these experiments. The in vivo experimental techniques employed in this study have been described in detail by Shah et al. (8).

TABLE I. Pesticides Tested in Present Study, with Specific Activity, Radiolabeling Position, and Source

Common name	Specific activity (mCi/mM)	Radiolabeling position	Source
Carbaryl	5.8	Naphthyl[1-^{14}C]	Amersham, Arlington Heights, IL
Carbofuran	39.4	^{14}C*(U)* Benzofuranyl ring	FMC Corp., Princeton, NJ
Chlordecone	3.2	^{14}C Ring*(U)*	Midwest Research Institute, Kansas City, MO
Dinoseb	4.29	^{14}C Ring*(U)*	Pathfinder Lab., Inc., St. Louis, MO
PCB[1]	9.2	2,4,5,2',4',5'-[^{14}C*(U)*]	New England Nuclear, Boston, MA

[1] 2,4,5,2',4',5'-Hexachlorobiphenyl.

Briefly, the animal's hair was clipped 24 h prior to dermal application with Oster animal hair clippers (Oster, Milwaukee, WI). Dosing solutions (0.1 mL young and 0.2 mL adult) containing radioactivity and appropriate dose (285 nmol/cm^2) in acetone were applied to the previously clipped mid-back region of the rat. The treated area, which represents approximately 2.3% of the total body surface area (2.8 cm^2 young , 5.6 cm^2 adult), was protected from grooming by placing a nonocclusive device over it. At the end of the designated time interval, the animal was anesthetized with ether and a blood specimen was obtained from the heart. Various tissues were removed to determine the distribution of dermally absorbed radio- labeled pesticides.

All the tissues, excretory products, and carcasses were analyzed for radioactivity by liquid scintillation counting as described by Shah et al. (8).

IN VITRO PROCEDURE

In vitro absorption of pesticides through young and adult skin was determined by using both Franz cells and continuous flow-through cells. The detailed experimental and skin preparation techniques have been described in detail by Shah et al. (9). Briefly, back hair was clipped 24 h prior to experiment. Animals were killed with carbon dioxide, and back skin was carefully excised. Skin was floated on tissue culture medium (minimum essential media, Eagle formula with Earl's Salts). Split thickness (350 μm thickness) skins obtained with a dermatome (Padgett Dermatome, Kansas City, MO) were mounted in the in vitro cells with epidermis exposed to the environment. Only one skin disc was used from each animal. The amount of pesticide (in acetone) per cm^2 area was kept the same for both in vivo and in vitro experiments. Receptor fluid was manually removed periodically from the Franz cells to determine the rate and extent of penetration, while continous flow through cells with a media flow rate of 3 mL/h were automatically sampled every 3 h. Sample preparation for radioactivity determination and oxidation of treated skin were done according to procedure described by Shah et al. (8). Briefly, the treated skin was combusted in the sample oxidizer to measure the remaining radioactivity in or on the skin. All other samples were mixed with 10 mL Insta-Gel (Packard Instrument Co., Downers Grove, IL) for scintillation counting.

Data Analysis

Statistical comparisons to determine the effect of age on skin penetration of pesticides were made using the Statistical Analysis System package (10) with age and age X time as class variables in the general linear model (GLM). Reciprocal variance was used for weighting. The significance of the laboratory method used (flow, static, in vivo) was determined with the SAS using age, method, and age X method, and age X method X time as class variables in the GLM and reciprocal variance for weighting.

A mathematical model was constructed along the lines of the physiological model developed by Bungay et al. (11,12). Least-squares fitting of the model to the data was done using SAAM 27, a computer package developed at the National Institutes of Health (NIH) by Berman and Weiss (13).

RESULTS

Skin absorption of carbofuran as determined by the three methods is shown in Table II. The in vivo skin absorption of carbofuran was 4.7% at 6 h and 33.5% at 72 h in young animals. In adults, it increased from 2.0% at 6 h to 11.8% at the 72 h time point. The static system gave 3.1% skin absorption at 6 h and 12.0% at 72 h in young animals, while in adults, absorption ranged from 1.7% at 6 h to 8.8% at the 72 h time point. The skin absorption of carbofuran as determined by the flow system in young animals ranged from 27.3% at the 6 h to 41.1% at 72 h time point. In adults, it ranged from 5.0% at 6 h to 11.2% at the 72 h time point. The young/adult ratio of skin penetration at 72 h was 2.9 (in vivo), 3.7 (flow), and 1.4 (static). Thus, all three methods gave higher skin penetration values in young rats compared to adults. However, the static cell gave consistently lower skin penetration values in young rats compared to in vivo observations.

Skin absorption of carbaryl as determined by the three methods is shown in Table III. The skin absorption of carbaryl (in vivo) ranged from 2.4% at 6 h to 38.5% at 72 h in young animals, and in adults it ranged from 3.6% at 6 h to 38.4% at 72 h. The skin absorption of carbaryl (static) ranged from 5.3% at 6 h to 20.9% at 72 h in the young, and in adults, it ranged from 4.7% at 6 h to 20.3% at 72 h. The skin absorption of carbaryl (flow) ranged from 18.8% at 6 h to 29.2% at 72 h in young animals, and in adults, it ranged from 8.1% at 6 h to 18.2% at 72 h. Both in vivo and static systems showed no significant young/adult differences in skin penetration of carbaryl at the 72 h time point, while the flow system gave significant differences in skin penetration between young and adult animals, with young absorbing more carbaryl compared to adults.

Skin absorption of dinoseb as determined by the three methods is shown in Table IV. Dinoseb showed rapid penetration in both young and adult rats compared to other pesticides. The results from the in vivo method showed a statistically significant lower skin absorption of dinoseb in young animals. In the young, skin absorption ranged from 11.1% at 1 h to 55.7% at 72 h, while in adults, it ranged from 22.3% at 1 h to 84.3% at 72 h. The static method indicates significant differences in skin penetration of dinoseb in young and adult, except at 1 h sampling period. In the young, the skin absorption value (static), for dinoseb ranged from 3.0% at 1 h to 74.9% at 72 h, and in the adult, it ranged from 1.5% at 1 h to 70.8% at 72 h. The flow system indicated significant young/adult differences in skin absorption of dinoseb at all time points, and young absorbed more than twice the amount of dinoseb as adults. The skin absorption value (flow) in young ranged from 6.4% at 1 h to 47.3% at 72 h, and 2.4% at 1 h to 20.6% at 72 h in the adult.

TABLE II. Methods Comparison of Skin Penetration of Carbofuran*

Time (h)	% Skin Penetration								
	Flow System			Static System			In vivo		
	Young	Adult	Young/ adult	Young	Adult	Young/ adult	Young	Adult	Young adult
6	27.3±4.5	5.0±0.6	5.5**	3.1±0.5	1.7±0.2	1.8	4.7±0.7	2.0±0.2	2.4
24	36.0±5.7	7.8±0.9	4.6**	7.0±0.8	4.6±0.5	1.5**	23.9±1.8	5.7±1.2	4.2**
48	39.4±6.0	9.7±0.9	4.1**	9.8±1.0	7.1±0.7	1.4**	26.1±2.6	6.8±0.1	3.8**
72	41.1±5.9	11.2±0.9	3.7**	12.0±1.1	8.8±0.8	1.4**	33.5±2.7	11.8±0.9	2.9**
All			4.3**			1.4**			3.4**
Age			**			**			**
Age X time			NS			**			**

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** Significant at $p < 0.05$ for the hypothesis of no young-adult difference.

NS Not significant, $p > 0.05$.

TABLE III. Methods Comparison of Skin Penetration of Carbaryl

	% Skin Penetration								
	Flow System			Static System			In Vivo		
Time (h)	Young	Adult	Young Adult	Young	Adult	Young Adult	Young	Adult	Young Adult
6	18.8±2.1	8.1±1.2	2.33*	5.3±0.5	4.7±0.6	1.15	2.4±0.3	3.6±0.5	0.66*
24	24.6±2.6	13.3±1.9	1.86*	11.4±0.8	11.1±1.1	1.02	14.9±2.5	15.2±1.2	0.98
48	27.2±2.8	16.0±2.3	1.70*	16.5±1.2	16.1±1.6	1.02	23.6±1.0	32.4±0.7	0.73*
72	29.2±2.9	18.2±2.6	1.60*	20.9±1.5	20.3±1.9	1.03	38.5±2.4	38.4±1.3	1.00
All			1.80*			1.04			0.89*
Age			*			NS			*
Age X Time			*			*			*

* Significant at $p < 0.05$ for the hypothesis of no young-adult difference.
NS Not significant, $p > 0.05$.

TABLE IV. Methods Comparison of Skin Penetration of Dinosob

	% Skin Penetration								
	Flow System			Static System			In Vivo		
Time (h)	Young	Adult	Ratio	Young	Adult	Ratio	Young	Adult	Ratio
1	6.4±1.0	2.4±0.6	2.64*	3.0±0.2	1.5±0.1	2.00*	11.1±2.1	22.3±8.8	0.50
6	29.0±3.5	11.19±2.2	2.61*	20.7±2.0	17.0±1.1	1.22	38.8±5.7	43.1±9.4	0.90
24	41.0±3.7	15.7±2.5	2.62*	59.1±7.4	52.3±3.1	1.13	63.0±2.9	85.5±1.8	0.74*
48	45.0±3.8	18.6±3.0	2.42*	70.9±8.4	66.2±3.8	1.07	55.3±9.7	85.1±1.9	0.65*
72	47.3±3.9	20.6±3.5	2.29*	74.9±8.0	70.8±3.8	1.06	55.7±2.4	84.3±2.7	0.66*
All			2.47*			1.10			0.70*
Age			*			*			*
Age X Time			*			*			*

* Significant at $p < 0.05$ for the hypothesis of no young-adult difference.

Methods comparison of hexachlorobiphenyl skin penetration in young and adult rats is shown in Table V. In vitro skin absorption values of hexachlorobiphenyl in young and adult rats were significantly lower than in vivo results. Skin absorption values in young at 6 h were 0.012%, 0.071%, and 2.5% as determined by flow, static, and in vivo methods, respectively. In adults, skin absorption values at 6 h were 0.015, 0.019, and 0.63% as determined by flow, static, and in vivo methods, respectively. Skin absorption values in young at 72 h were 1.25, 2.50, and 33.2% as determined by flow, static and in vivo methods, respectively. In adults, 72 h skin absorption values were 1.36, 1.15, and 19.6% as determined by flow, static and in vivo methods, respectively. Both in vitro methods showed nonsignificant differences in skin penetration of hexachlorobiphenyl between young and adult rats, while in vivo results showed significant differences in skin penetration between young and adult rats at all time points.

Methods comparison of skin penetration of chlordecone (Kepone) is shown in Table VI. In vitro skin absorption values of chlordecone in young and adult rats were significantly lower than in vivo values. Skin absorption values in young at 6 h were 0.45, 0.022, and 0.51% as determined by flow, static, and in vivo methods, respectively. In adults, skin absorption values at 6 h were 0.052, 0.026, and 0.398% as determined by flow, static, and in vivo methods, respectively. Skin absorption values in young at 72 h were 1.4, 2.2, and 9.1% as determined by flow, static and in vivo methods, respectively. In adults, 72 h skin absorption values were 1.2, 2.2, and 7.9% as determined by flow, static, and in vivo methods, respectively. All three methods showed higher skin penetration of chlordecone in young compared to adult animals.

PHYSIOLOGICAL MODELING

A physiological compartmental model for carbofuran dermal absorption and disposition is shown in Figure 1. The compartmental volumes (V_T) were the average weight of kidney, liver, and body found in the present study. The other compartmental volumes were derived from the body proportions given by Lutz et al. (14). Cardiac output and blood flow rates were scaled directly with compartmental size from the values given by Bungay et al. (11). A blood flow rate for a 1.9 g kidney was calculated to be 8.33 mL/min from a plasma flow rate of 5 mL/min and hematocrit of 40% (15).

The system of differential equations describing the transfer of material between organ compartments is given below. For blood

$$\frac{d}{dt}y_B = \sum_{\substack{i=K,L\\M,S}} \frac{Q_i}{V_i R_i} y_i - \frac{Q_i}{V_B} y_B + k_a y_t$$

For muscle and nontreated skin

$$\frac{d}{dt}y_i = \frac{Q_i}{V_B} y_B - \frac{Q_i}{V_i R_i} y_i \qquad \text{where } i = M,S.$$

Table V. Methods Comparison of Skin Penetration of 2,2',4,4',5,5'-Hexachlorobiphenyl

	% Skin Penetration								
	Flow System			Static System			In vivo		
Time (h)	Young	Adult	Young/ adult	Young	Adult	Young/ adult	Young	Adult	Young adult
6	0.012±0.003	0.015±0.002	0.83	0.071±0.022	0.019±0.005	3.63	2.50±0.20	0.63±0.06	3.94*
24	0.17±0.04	0.17±0.03	1.00	0.46±0.12	0.188±0.054	2.43	13.62±0.53	8.35±0.53	1.63*
48	0.60±0.13	0.58±0.08	1.05	1.25±0.24	0.592±0.138	2.11	28.1±3.5	18.4±0.6	1.53*
72	1.25±0.23	1.36±0.16	0.92	2.50±0.32	1.15±0.21	2.18	33.2±2.2	19.6±1.5	1.69*
All			0.96			2.20			1.65*
Age			NS			*			*
Age X time			*			*			*

* Significant at $p < 0.05$ for the hypothesis of no young-adult difference.
NS Not significant, $p > 0.05$.

Table VI. Methods Comparison of Skin Penetration of Chlordecone

Time (h)	% Skin Penetration								
	Flow System		Young Adult	Static System		Young Adult	In Vivo Method		Young Adult
	Young	Adult		Young	Adult		Young	Adult	
6	0.450±0.043	0.052±0.020	8.69*	0.022±0.007	0.026±0.007	0.86	0.513±0.063	3.98±0.108	1.29
24	0.921±0.052	0.205±0.090	4.49*	2.47±0.072	0.199±0.039	1.24	3.570±0.453	2.508±0.606	1.42
48	1.201±0.057	0.699±0.243	1.80*	1.100±0.238	0.912±0.161	1.21	6.825±0.219	5.626±0.686	1.21
72	1.398±0.057	1.234±0.310	1.13*	2.213±0.435	2.153±0.320	1.03	9.066±0.664	7.922±1.061	1.14
All			1.84*			1.09			1.21

* Significant at $p < 0.05$ for the hypothesis of no young-adult difference.

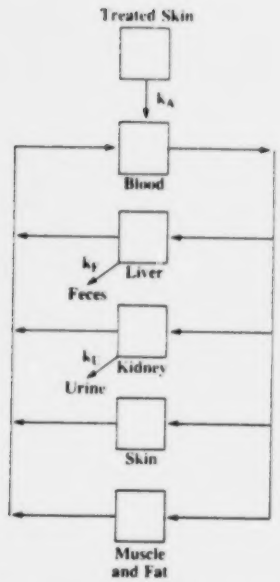

Figure 1. Physiological model for carbofuran dermal absorption and disposition in the rat. (Reproduced with permission from ref. 9. Copyright 1987 Hemisphere Publishing Corporation.)

For kidney

$$\frac{d}{dt}y_K = \frac{Q_K}{V_B}y_B - \frac{Q_K}{V_K R_K}y_K - k_U y_K$$

and for liver

$$\frac{d}{dt}y_L = \frac{Q_L}{V_B}y_B - \frac{Q_L}{V_L R_L}y_L - k_F y_L$$

For treated skin

$$\frac{d}{dt}y_T = -k_a y_T, \quad y_T(0) = D_0$$

Nomenclature

D_0 Dermal dose (n moles)
Y_i Radioactivity in compartment i (n moles)
Q_i Blood flow rate to tissue i: (mL/h)
V_i Volume for tissue compartment i: (mL)
R_i Equilibrum tissue/blood ratio for compartment i
k_A Dermal absorption rate constant (h^{-1})
k_U Urinary excretion rate constant (h^{-1})
k_F Fecal excretion rate constant (h^{-1})

Subscripts

B Blood
K Kidneys
L Liver
M Muscle
S Nontreated skin
T Treated skin
F Feces
U Urine

Carbofuran levels from blood, kidney, liver, skin, carcass, urine, and feces were fitted to the model using SAAM27 to optimize the parameters for equilibrum tissue/blood ratio (R_T), the urine (K_U), and feces (K_F), excretion-rate constant, and the dermal absorption-rate constant (K_A). The parameter values are shown in Table VII.

The dermal absorption-rate constant (K_A) for young, 0.00548 h^{-1}, is about 3 times that for adults, 0.00173 h^{-1}. The model results for dermal absorption were in agreement with the observed values after 24 h for both young and adult rats. Before 24 h, the model results were approximately half of the observed values, perhaps indicating an additional rapid component of absorption which was not incorporated in the model. The urinary excretion rate constants (K_U) were found to be 36.1 h^{-1} for young and 26.1 h^{-1} for the adult. The fecal excretion rate constants (K_F) were found to be 0.67 h^{-1} for young and 0.24 h^{-1} for the adult. The increased dermal absorption-rate constant in young compared to adult animals appears to be balanced by the increased excretion-rate in the young, such that the body burdens in young and adults are about the same at 120 h.

The equilibrium tissue-to-blood ratios (Table VII) for adult animals were higher than those of the respective organs in the young. Most notable is the difference in the kidney-to-blood ratio of 10.3 for adults as compared to 5.4 for young, indicating the greater sequestering of carbofuran-derived radioactivity in adults than in young.

TABLE VII. Parameters[a] Employed in the Physiologic Model for Carbofuran Absorption and Disposition in the Young and Adult Rat*

Rat	Organ	V_i(mL)	Q_i (mL/min)	R_T
Adult	Blood	14.18 (9)[b]		39.4010.29
	Kidneys	1.31[b]		5.782.47
	Liver	5.93[b]		9.491.94
	Skin	25.21 (16)		3.153.65
	Muscle and fat	89.82 (57)		4.98
	Body	154.58[c]		
	k_U = 26.09 h^{-1}	K_F = 0.239 h^{-1}		K_A = 0.00173 h^{-1}
Young	Blood	6.38 (9)b		17.74
	Kidneys	0.72[b]		3.195.41
	Liver	3.03[b]		4.861.91
	Skin	11.35 (16)		1.421.28
	Muscle and fat	40.44 (57)		2.242.38
	Body	70.94[c]		
	K_U = 36.06h^{-1}	K_F = 0.674 h^{-1}		K_A = 0.00548 h^{-1}

[a] V_i, compartmental volume; Q_i, blood flow rate to the organs; R_t, equilibrium tissue to blood ratio; K_U, rate constant for urinary excretion; K_F, rate constant for fecal excretion; K_A, rate constant for dermal absorption.

[b] Percent of body weight given in parentheses.

[c] Observed.

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A steady state was reached rapidly. The model predicted organ content to be maximum at 6 h, followed by a decrease in content as a result of the decline in the amount that was available for penetration. Continuous exposure was simulated with the model by maintaining a unit constant amount on the skin. Equilibrium levels calculated in the organs as fractions of the amount on the skin for young and adult, respectively, were 1.5 X 10^{-4}, and 0.65 X 10^{-4} for kidney, 3.7 X 10^{-4} and 1.4 X 10^{-4} for liver, 9.4 X 10^{-4} and 4.7 X 10^{-4} for skin, and 81 X 10^{-4} and 40 X 10^{-4} for the total body. Corresponding excretion rates (fraction/h) for young and adults were 52 X 10^{-4} and 17 X 10^{-4} h^{-1} for urine and 2.5 X 10^{-4} h^{-1} and 0.34 X 10^{-4} h^{-1} for feces. From this temporal experiment with a single applied dose of carbofuran, the linear compartmental flow model provides a good fit to the data. A more complete validation of the model would require data at a sequence of doses as well as data from multiple dose experiments.

For chlordecone, a model developed from intravenous (iv) dosing was used but modified to accommodate the dermal absorption pathway. A time-varying dermal absorption rate constant was found to enhance the model fit. The urinary excretion rate constant was also time-dependent. Excretion began at about 0.75 h^{-1} and finished at about 0.01 h^{-1} at 120 h. Equilibrium tissue/blood ratios agreed with those found by iv dosing except for skin. Skin was found to have an equilibrium tissue/blood ratio of about 3.5 instead of 6 as determined by iv dosing. This was the lowest ratio for any tissue. The highest ratio was 10 which occurred for liver.

Dermal absorption for dinoseb was biphasic requiring two first order compartments to adequately simulate the observed data. Tissue/blood ratios were lowest in muscle, about 0.15, and highest in kidney, about 0.55. Since all ratios were less than 1.0, no sequestering of dinoseb radioactivity is indicated. The urinary pathway was the preferred route of excretion by about a 4:1 ratio as compared to the fecal route.

Hexachlorobiphenyl was modeled with separate compartments for parent and metabolite as determined from published iv experiments (14). Dermal absorption was described by a single first order compartment but delayed by about 1 h in young rats and 4 h in adult rats. The tissue/blood ratio for adipose for the parent compound was found to be in the 100's or 1000's indicating a very small release of parent from the adipose compartment. Muscle had the lowest tissue/blood ratio for parent of about 4. The metabolism constant for liver was about 0.02 mL/min which is quite slow. The metabolites are readily excreted and produced tissue blood ratios of only 1 to 4.

DISCUSSION:

A number of studies have suggested higher skin permeability in premature and full-term infants compared to adults. Behl et al. (4) and Maibach and Boisits (16) have reviewed the literature on

age-related percutaneous absorption. Behl et al. (4,17) reported in vitro skin penetration of water, methanol, ethanol, butanol, hexanol, and octanol in male hairless mice of different ages. They observed increased skin permeabilities reaching maximum values around 25 days of age, then declining sharply up to about 60 days of age, and remaining more or less invariant thereafter.

In the present study, age-related differences in percutaneous absorption of some of the pesticides tested were observed. In many cases significant influence of methods employed to determine the skin absorption of these pesticides was also observed. Results at 72 h from the in vivo method showed age-related differences in skin penetration for carbofuran, dinoseb, and hexachlorobiphenyl. The flow method showed statistically significant skin penetration differences for dinoseb, carbaryl, carbofuran and hexachlorobiphenyl. The static method showed significant young/adult penetration differences only for carbofuran and hexachlorobiphenyl. This study clearly demonstrates that significant differences in absolute penetration values arise from the different methods used.

A number of studies have shown good correlation between in vivo and in vitro skin permeability values for a number of chemicals and animal species because skin absorption has been considered as the passive diffusion process through a rate-limiting, nonliving barrier, stratum corneum. Franz (18) compared the percutaneous absorption (in vitro) of 12 organic compounds with in vivo results reported by Feldmann and Maibach (19). He reported excellent qualitative agreement between in vitro and in vivo methods. He further reported the discrepancies in the quantitative agreement between the two sets of data. Later on Franz (20) reported that discrepancies in quantitative agreement was due simply to experimental variation based on additional in vitro experiments to simulate in vivo conditions by washing skin after 24 h. Bronaugh (21) reported identical in vivo and in vitro rat skin absorption values for benzoic acid, acetylsalicylic acid and urea.

The two in vitro methods utilized here, namely, the static and flow-through diffusion cells, have been recently compared by Bronaugh and Stewart (22). The authors (22) reported the comparative skin absorption values for water, cortisone, and benzoic acid through rat skin as determined by static and flow-through diffusion cells. They reported similar absorption profiles and quantitative values between the two diffusion systems for water, cortisone, and benzoic acid under the experimental conditions described. However, discrepancies in absorption values were observed for a hydrophobic compound, [3-phenyl-2-propenyl-2-aminobenzoate (cinnamyl anthranilate)] between the two diffusion cell systems; the flow-through diffusion cell system gave enhanced absorption value for cinnamyl anthranilate compared to the static cell system. They have further reported good agreement between in vivo and in vitro (static as well as flow-through diffusion cells) skin permeation for cortisone and benzoic acid when each was applied in petrolatum vehicle.

We have reported discrepancies in the skin absorption values as determined by the two in vitro methods. We also observed poor

correlation between in vivo and in vitro results for these highly complex pesticidal chemicals. There are a number of factors that can influence the skin absorption measurements. The influence of receptor fluid for in vitro skin absorption measurements has been widely documented. For example, the in vitro absorption of chlordecone and hexachlorobiphenyl in the present study never exceeded more than than 2.5% in both diffusion cell systems. These two chemicals have poor water solubility (0.9 ppb for hexachlorobiphenyl) and the receptor fluid utilized in this investigation may not be sufficiently lipohilic to remove the chemical from the dermis (split-thickness skin). Bronaugh and Stewart (23) suggested that the lack of solubility of the hydrophobic penetrant into the receptor fluid for in vitro methods could be a contributing factor accounting for in vivo and in vitro discrepancies in skin absorption values. Bronaugh and Stewart (23) reported maximum absorption of cinnamyl anthranilate by using 6% PEG-20 oleyl ether receptor fluid with either type of diffusion cell system.

Recently, Scott and Ramsey (24) reported that the highest absorption of cypermethrin was achieved when 50% aqueous ethanol was used as the receptor fluid which was in agreement with in vivo absorption through the rat skin. They further reported that the absorption of cypermethrin measured with two other receptor fluids (6% Volpo 20 and 20% fetal calf serum) was very similar to 50% aqueous ethanol as the receptor fluid. Thus the above mentioned study suggests that, the solubility of the penetrant into receptor fluid, rather than the diffusion of penetrant through the epidermal membrane, is the rate limiting step.

It appears that the results obtained by different methods have to be carefully evaluated with the experimental conditions prescribed. It is also clear from the literature that the in vitro results can be influenced by a number of factors. Perhaps more comparative studies may improve the understanding of the in vitro and in vivo correlation. Validation of recently recommended guidelines for in vitro percutaneous penetration studies (25) with a number of chemicals may help in understanding the in vitro skin absorption process.

Utility of pharmacokinetic models has been explored for extrapolation to man, animal species scaling, extrapolation of results beyond the observed time point, simulation of continuous or multiple exposure situation, tissue dose extrapolation, improving experimental design, and overall reduction of animal usage. Physiological models have been shown to be adequate for other chemicals (26,27). The applications of physiologically based modeling and its technique has been reviewed by a number of investigators (26,28,29). In the present study, physiologically-based pharmacokinetic models were used to decribe dermal absorption and disposition processes for the chemicals studied. Rate constants for dermal absorption, and urinary and fecal excretion, as well as tissue to blood steady state ratios were determined.

A physiological model describing carbaryl radioactivity was fitted to the tissue distribution data. The dermal absorption was found to be described by a single first order compartment for both

young and adult rats over the experimental period. The order of the equilibrium tissue/blood ratios was the same in young and adult rats. The tissue with the largest tissue/blood ratio of about 6 or 7 was kidney. The untreated skin has the lowest equilibrium tissue/blood ratio of about 0.8 to 1.0. The rate constant for kidney to urine excretion was large at 15 to 20 h^{-1}.

A more complex model to include metabolism, blood clearance, release of chemical from solvent to skin, incorporation of viable layer, and metabolism during absorption, etc., may aid in improving the model's utility.

CONCLUSIONS:

Percutaneous absorption was compared in young and adult Fisher 344 rats for five pesticides utilizing both in vivo and in vitro methods. In vivo penetration of the pesticides during a 72 h period, ranged from approximately 8.0% (chlordecone, adult) to 84% (dinoseb, adult), depending on compound and age of the animal. Three pesticides, (carbofuran, hexachlorobiphenyl, and chlordecone) showed higher skin penetration values in young compared to adult. Differences in skin penetration values were observed among pesticides due to methods of measurement. Two in vitro methods, namely, static cell and flow-through diffusion cell systems gave different penetration values. A physiological compartmental model appears to be good for simulating dermal absorption and disposition of the pesticides studied.

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